APPENDIX 2:

EXPERIMENTAL PROTOCOLS

A. Methods Used In Diabetic Neuropathy Model

Induction of diabetes

Following an overnight fast, male CD-COBS rats weighing 200-250 g were made diabetic by a single i.p. injection of streptozotocin (STZ; 60 mg/kg body weight). Urine was tested two days later to confirm that the mice became hyperglycemic following treatment. Rats with glucose levels of >280 mmol/l (>5%) were considered diabetic. Rats were randomized and housed at 2-3 per cage. Control animals were age-matched and saline-treated (rather than STZ).

EPO Treatment

Diabetic and control groups of rats receiving EPO injections: 5000 units/kg b.w. i.p. three times a week.

Nociceptive thresholds

Thermal nociceptive threshold to radiant heat was quantified using the paw withdrawal in the hot-plate test (Woolfe and MacDonald, 1944). The hot plate was placed under a 25 cm high Plexiglas cylinder, and temperature was maintained at 50°C as a control for untreated rats in latencies of about 10 sec. The paw withdrawal latency was defined as the time between placement of the rat on the hot plate and the time of withdrawal and licking of hind paw. The test was performed on week 3.5, 4.5, 5.5 and 6.5, and each animal was tested for two trials at 30 min. time-distance each.

The mechanical nociceptive threshold was quantified using an Analgesy-meter (Ugo Basile Italy). This instrument generates a mechanical force that is applied to the dorsal surface of hind paw, via a cone-shaped plunger, which increases linearly with time. The mechanical nociceptive threshold was defined as the force at which the rat attempts to withdraw the paw (force is in grams and cut-off force is 250 g). Rats were trained the previous 3 days prior to performing the tests. Each animal was tested for 3 trials each time, and results are mean of these measurements.

Nerve Conduction Velocity

The antidromic nerve conduction in the tail nerve was assessed using a Myto EBNeuro electromiograph by placing recording ring electrodes distally in the tail, while a stimulating ring electrode was placed 5 cm and 10 cm proximally with respect to the recording point. The latency of potential recorded in the two sites after nerve stimulation (stimulus duration 100 msec, filter 1 Hz – 5 MHz) was determined (peak to peak) and nerve conduction velocity was calculated accordingly.

Statistical Analysis

The statistical comparison (NCV, thermal and mechanical threshold was performed with an analysis of variance (one-way ANOVA) using the Tukey-Kramer post-test (significance at $p<0.05$) and/or Student's t-test where appropriate.

B. Methods Used In The SOD Model

Stride Length Measurement

The stride measurement test was done in accordance with de Medinaceli *et al*, 1982, Exp. Neurol. 77:634-643). Paralysis of hindlimbs was measured by dipping the hindpaws of the animals in ink and the animals were permitted to walk over a strip of paper in a runway and the average stride length for each animal was measured.

Extension Reflex Deficit Measurement

The extension reflex was done in accordance with Barnéoud and Curet (1999). A score of 2 corresponded to a normal extension reflex of both hindpaws, a score of 1 to the extension relex of only one hindpaw, and a score of 0 to the absence of any hidlimb extension.

Latency on Rotating Bar Measurement

Motor function was evaluated using the rotarod test, as described previously (Bendotti *et al.*, 2001). Briefly, mice were placed on a rotating rod ("rotarod") at 15 r.p.m. The time each mouse remained on the rod was recorded. If the mouse remained on the rod for 3 mins., the test was stopped and scored as 3 mins. The test was perforemed twice and only the maximum time wastaken into account. The onset of motor deficits was define as the first day a mouse could not remain on the rotarod for 3 min.